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Effect of Different Processing Techniques on Protein Quality of Hatchery Waste Meals

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Abstract.- The nutrient composition and protein quality of hatchery waste (HW) was evaluated by using different processing techniques *i.e.*, cooking, autoclaving and extrusion. The protein contents of the cooked, autoclaved and extruded hatchery waste meals were 43.67, 44.10 and 41.64%, respectively. Microbial analysis of the raw HW exhibited high microbial counts. Different processing techniques reduced the microbial count of HW. Autoclaving reduced both the total viable count (TVC) and total coliform count (TCC) to the minimum compared to other heat treatments. Protein quality of cooked, autoclaved and extruded HWM was measured in terms of protein efficiency ratio (PER) and net protein utilization (NPU). The weight gain in group of broilers consuming reference diet (casein) as sole source of protein was significantly ($P \leq 0.05$) higher compared to the other experimental groups. The PER results from all processing techniques along with NPU data supported an overall conclusion that processing HW with cooking and autoclaving is comparable in terms of NPU. Autoclaving proved more beneficial in terms of PER. But overall values of PER and NPU revealed that processing of HWM can generate nutrient rich, palatable product that was comparable to the traditional feed ingredients.

Key words: Hatchery waste, microbial count, NPU, PER, protein quality.

INTRODUCTION

Poultry population (commercial as well as indigenous) is increasing and there is proportionate reduction in the availability of feed resources, especially animal protein (Jatoi *et al.*, 2014). The quality of different animal protein sources like fish meal, poultry meal and blood meal is too variable to be dependable and have some reservation on the part of nutritionists. This situation has necessitated using non-conventional feedstuffs as replacement for the conventional ones (Attah and Ologbenla, 1993). Many of such wastes, particularly hatchery waste, if managed and processed appropriately, have the potential for increasing the availability of an alternative source for poultry feed. About 140,000 tons of waste is produced annually in the United States alone by hatcheries which produce commercial broilers, laying hens and turkeys Das *et al.* (2002). While in Pakistan, in the year 2008-09, the eggs set for incubation produced 9,974 t of HW (GOP, 2008-09). Hatchery waste includes infertile eggs, dead embryos, egg shells from hatchings, and unsalable chicks (Freeman, 2007). As the poultry industry will continue to expand, increase in on farm waste material and hatchery residue necessitates nutritionists to search for new efficient ways for conversion of these materials into useful products (Blake and Donald, 1992).

The disposal of hatchery waste is of great concern for poultry industry. The common ways of disposal are incineration, rendering as well as land filling (Miller, 1984). These methods of disposal are not only costly, rather high moisture content of the raw material create pollution for the environment (Vandepopuliere *et al.*, 1977). Composition of hatchery waste indicates that by proper processing it can be converted into nutritionally dense meal. Quality of meals of animal origin can vary depending upon processing technique (Johnston and Coon, 1979). During processing, protein quality of meal of animal origin can be affected by temperature and pressure. Batterham *et al.* (1986) reported that an increase in processing temperature (from 125°C to 150°C) can cause reduction in lysine availability (from 86 to 35%). Tadtiyanant *et al.* (1993) recommended that to generate good quality product, composting or rendering can be adopted separately or in combination. Hatchery by-products can be re-utilized as a new non-conventional feed

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stuff in to poultry ration by proper processing Babiker *et al.* (1991). It has potential to replace fish meal. The practice of disposing the hatchery waste as garbage at far flung areas is not only the wastage of valuable protein and energy sources; rather a contributing factor to the environmental pollution. The objective of the present study was to recycle hatchery waste by using different processing techniques and to determine the protein quality of different processed HWMs.

MATERIALS AND METHODS

Processing of hatchery waste meals

The raw hatchery waste (HW) comprising infertile eggs, shells, dead in shells and low grade unsalable chicks were subjected to the following three processing techniques, *viz.*, cooking, autoclaving and extrusion (Mahmud *et al*., 2015).

After drying the representative samples of the HWM prepared from the above mentioned processing techniques were subjected to chemical, microbial, amino acids and minerals analyses according to (AOAC, 2000).

Table I.- Composition of experimental rations for the determination of protein quality.

Ingredients	A	B	C	D	E
Corn starch	73	54.7	24.6	28.6	29.7
Glucose	17	23.5	6.6	9	9.4
Vitamin mixture	1	1	1	1	1
Mineral mixture	4	5.5	3.4	3.8	3.8
Casein	0	10	0	0	0
Cotton seed oil	5	5.3	4.7	5	5
HW (cooked)	0	0	0	52.6	0
HW (autoclaved)	0	0	0	0	51.1
HW (extruded)	0	0	59.7	0	0
Total	**100**	**100**	**100**	**100**	**100**

Vitamin mixture per 100g basal diet contained: Vit, A 1500 IU; Vit. D_3 200 IU; Vit. E 10 IU; Vit. K 0.5 mg; Biotin 0.15 mg; Folacin 0.55 mg; Thiamine 1.80 mg; Pyridoxine 3.5 mg; Riboflavin 3.6 mg; Niacin 35 mg; calcium pantothenate 3 mg; Cyanocobalamine 2 µg; Choline chloride 0.15 g.

**Mineral mixture per 100g basal diet contained $K_2H_2PO_4$ 1.61; $MgSO_4.7H_2O$ 0.51g; $MnSO_4.4H_2O$ 0.25 g; NaCI 0.837g; $FeSO_4$ 0.137g; KI 0.004 g; $ZnCI_2$ 1.5 mg; $CuSO_4$. $5H_2O$ 1.5 mg; $Ca(H_2PO_4)$ 2.5 g and $CaCO_3$ 2 g.

Estimation of protein quality

Protein quality of cooked, autoclaved and extruded HWM was evaluated in terms of protein efficiency ratio (PER) and net protein utilization (NPU). For this purpose, a ten days feeding trial was conducted to determine the protein quality of cooked, autoclaved and extruded HWM. Five poultry rations were formulated according to the standards prescribed by (NRC, 1994) for broiler chicks. Ration "A" was protein free, meeting all other nutritional requirements of the birds. Ration "B" was used as reference diet for experimental birds and contained casein as sole source of protein. Ration C, D and E had extruded, autoclaved and cooked HWMs, respectively as exclusive sources of protein. The composition of each ration is given in (Table I). Twenty five straight run 14-days old broiler (Hubbard) chicks were divided randomly into five groups in such a way that there were five chicks in each group. Each group was divided randomly into five experimental units in such a way that each chick represented as single replicate. All the birds were weighed at the start of the experiment. Five experimental rations were allocated to each group and there were five chicks on each ration. Clean fresh water and feed were offered *ad libitum* to each bird throughout the experimental duration. The room temperature was maintained at 28±1°C. The daily feed offered, refusal and intake was recorded. The birds were weighed daily and weight gain was also recorded. Feces of each bird were collected daily in a separate sterilized plastic bottle containing 2% sulphuric acid. Faeces were dried in the oven at 70°C till constant weight. At the end of the feeding trial, the birds in each group were anesthetized with chloroform, their cranial as well as abdominal cavities were opened and weighed before and after drying at 105°C to a constant weight. The dried carcass were ground and analyzed chemically for nitrogen content. Similarly, the faeces of each bird were also chemically analyzed for nitrogen content. Net protein utilization (NPU) and protein efficiency ratio (PER) were worked out by using the formula of (Miller and Bender, 1955) as under:

$$\text{Net protein utilization (NPU)} = \frac{B - (B_k - I_k)}{I}$$

Where B, total body nitrogen of chicks on test diets; B_k, total body nitrogen of chicks on protein free

diets; I, nitrogen intake of chicks on test diets; I_k, nitrogen intake of chicks on protein-free diet.

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Body weight gain (g)}}{\text{Protein consumed (g)}}$$

Statistical analyses

The data were statistically analyzed through analysis of variance technique under complete randomized design (Steel *et al.*, 1997). Means were compared for significance of difference with Duncan Multiple Range Test (Duncan, 1955) to deduce the results.

RESULTS

The chemical and microbial analyses of processed HWM by using different processing techniques are depicted in Tables II and III, respectively.

Protein quality

Average weight gain, feed intake, protein efficiency ratio (PER) and net protein utilization (NPU) for various rations have been presented in Table IV.

Weight gain

Average weight gain in 10 days trial in birds fed diets containing casein (standard), cooked, autoclaved and extruded HWMs were 86.50, 76.74, 74.66 and 72.22 g, respectively. Maximum weight gain was observed in birds fed casein diet. The data regarding weight gain showed significant ($P \leq 0.05$) differences among the groups. The comparison of means revealed significantly ($P \leq 0.05$) higher weight gain in case of casein diet as compared to diets containing cooked (76.74g), autoclaved (74.66g) and extruded (72.22g) HWMs. Significantly ($P \leq 0.05$) lower (72.22g) weight gain was observed in group fed extruded HWM as compared to diets containing cooked, autoclaved HWMs and casein. Non-significant ($P > 0.05$) difference was found between diets containing autoclaved and cooked HWMs.

Feed intake

The data on feed intake of birds fed experimental diets revealed that the groups fed on casein (230g) and cooked (227g) HWM were significantly ($P \leq 0.05$) different as compared to extruded (226g) and autoclaved (222g) HWM diets. Non-significant ($P > 0.05$) difference was found between the diets containing casein and cooked HWM as well as between the groups fed on extruded and autoclaved HWMs.

Table II.- Chemical analysis of raw, cooked, autoclaved and extruded HWM on dry matter basis.

Nutrient %	Raw hatchery waste (Control)	Cooked HWM	Autoclaved HWM	Extruded HWM
Crude protein	44.63	43.67	44.10	41.64
Crude fat	26.46	27.14	25.35	26.85
Crude fiber	1.05	1.62	1.31	1.47
Total ash	25.88	25.81	26.94	27.90
Nitrogen free extract	1.98	1.76	2.30	2.14
Calcium	17.56	19.02	18.62	18.95
Phosphorus	1.63	1.99	1.44	1.54

HWM, hatchery waste meal; HW, hatchery waste; NFE, nitrogen free extract

Table III.- Microbial count and bacterial species identified in different processed HWMs.

Treatment	TVC	TCC	Species identified
Raw HW	8.3×10^{7a}	1.9×10^{5a}	*Salmonella, E. coli, Bacillus, Streptococuss Pasteurella, Klebsheilla*
Extruded HW	3.7×10^{3b}	2.9×10^{2b}	*E. coli*
Cooked HW	1.9×10^{5c}	2.4×10^{4c}	*Bacillus* sp.
Autoclaved HW	4.7×10^{3d}	3.0×10^{2b}	*E. coli*

Different superscripts on means in a column show significant difference ($P \leq 0.05$)
TVC, Total viable count; TCC, Total coliform count

Protein efficiency ratio (PER)

The PER values of cooked (1.46), autoclaved (1.50) and extruded HWM (1.38) were less than that of casein (standard) diet (1.63). The statistical analysis of data revealed that PER values of all

Table IV.- Average weight gain, feed intake, protein intake, PER and NPU values of HWM.

Source	Weight gain (g)	Feed consumed (g)	FCR	Protein intake (g)	PER	NPU
Casein	86.50±7.21^{c}	230.00±21.50^{b}	2.66±0.02^{a}	52.90±6.12	1.63±0.01^{d}	74.22±0.02^{c}
Cooked HWM	76.74±3.88^{b}	227.00±14.38^{b}	2.95±0.03^{b}	52.21±6.04	1.46±0.01^{b}	45.71±0.01^{b}
Autoclaved HWM	74.66±5.62^{b}	222.52±19.91^{a}	2.98±0.01^{b}	51.18±6.17	1.50±0.01^{c}	45.22±0.01^{b}
Extruded HWM	72.22±6.34^{a}	226.17±12.28^{a}	3.13±0.02^{c}	52.00±6.20	1.38±0.01^{a}	40.63±0.01^{a}

Different superscripts on means in a column show significant difference ($P \leq 0.05$).
PER, Protein efficiency ratio; NPU, Net protein utilization; HWM, Hatchery waste meal.

protein sources tested, differed significantly ($P \leq 0.05$) among all groups. It was observed that casein gave maximum (1.63) PER value, which was significantly ($P \leq 0.05$) higher than those of cooked, autoclaved and extruded HWMs, while minimum (1.38) PER value was observed in group fed on extruded HWM.

Net protein utilization (NPU)

The NPU values of cooked (45.71), autoclaved (45.22) and extruded HWM_s (40.63) were less than that of casein based diet (74.22). The statistical analysis of data revealed that NPU value of diet containing casein was significantly different ($P \leq 0.05$) from rest of the three diets. It was also observed that diets fed on autoclaved and cooked HWMs showed significant ($P \leq 0.05$) difference with that of extruded HWM. However, there was non-significant ($P > 0.05$) difference between autoclaved and cooked HWMs.

Microbial analysis

Microbial analysis using total plate count (in colony forming units) was done for raw as well as processed HWMs. Total viable bacterial count and species present in raw, processed HW meals are presented in Table III. Total viable count (TVC) and total coliform count (TCC) for raw HW were 8.3×10^7 and 1.9×10^5, respectively. Most prevalent species were *Salmonella* and *E. coli*. All types of processing techniques were found efficient in counter acting TCC as there was non significant ($P > 0.05$) in TCC of processed meals. Extrusion was most effective in reducing TCC as compared to cooked and autoclaved. Similarly, autoclaved significantly reduced ($P > 0.05$) TCC when compared to cooked meal but TCC in all samples were in safe limit.

Both autoclaving and extrusion were found quite efficient in reducing TVC and TCC. Although the TVC and TCC were higher in cooked HWM as compared to autoclaved and extruded, but even it was under safe limit. When data for microbial count was statistically compared for differences, the processing techniques significantly ($P \leq 0.05$) affected the TVC as well as TCC (Table III). When treatments were compared among themselves irrespective of the raw HW, significant ($P \leq 0.05$) differences were found between different processing techniques for TVC and non-significant ($P > 0.05$) differences were observed for TCC.

DISCUSSION

The processing techniques used in the study revealed that protein content of the HWM depends upon the composition of the waste. The cooked, autoclaved and extruded meals CP were 43.67, 44.10, and 41.64%, respectively. The meals prepared in the present study were comparable to that of Saima *et al.* (2003) who reported 43.10 % and 42.99% CP in cooked and toasted HWM, respectively. However, (Ristic and Kormanjos, 1988) revealed 22.4% CP in autoclaved HWM. Less CP in their study might be due to high shell moiety. Different factors like hatching percentage, species and shells can affect the composition of meal. Separation of shells from meal to enhance protein percentage is a common practice. In the present study the CP content of extruded HW was somewhat less than autoclaved HWM. This might be due to the reason that HW was extruded without blending. While Lilburn *et al.* (1997) found more CP content *i.e.*, 44.6% in extruded as compared to

22.2% CP in autoclaved HW with 70% less lysine. This was due to the reason that hatchery residue used for both processing techniques was collected on separate day. The make up of the product on two sampling days could have been different. Same situation was observed in ash content. In the present study, ash contents were 25.81, 26.94 and 27.90% for cooked, autoclaved and extruded HWM, respectively. Ilian and Salman (1986) reported 60.4 % and Rasool *et al.* (1999) found only 14.04 % ash in HWM. This large variation in ash composition may also be attributed to above mentioned factors. The concentration of calcium and phosphorus level is directly related with ash content. So, variation in these nutrients would change ash content.

There were significantly high viable count in raw hatchery waste and it contained large number of pathogenic bacteria. Due to this reason, the raw HW cannot be included in the poultry diet as such. In the present study, the processing techniques did not eliminate the viable count completely but managed it to safe level. In this regard all techniques were found to be efficient with extrusion at the top. Tadiyanant *et al.* (1993) in a comparative study of dead turkeys and hatchery solid found that standard plate counts of pre-extrusion blended mixtures before extruding ranged from $3.2x10^4$ cfu/g to $2.5x10^{10}$ cfu/g respectively. However, just after processing by extrusion, no aerobic micro-organisms were observed in any of the products when analyzed. They inferred that high temperature short time extrusion was excellent for ingredient processing and eliminating aerobic micro-organisms.

Results of the present study are in line with findings of Haque *et al.* (1991) who determined total number of aerobic micro-organism present in unextruded poultry by-product meal diet to be 47000 cfu/g which can completely be eliminated by high temperature and short time extrusion process. (Miller, 1984) reported that when hatchery wastes were processed through high temperature extrusion, no *Salmonella* organisms were found. Dhaliwal *et al.* (1996) concluded that *Bacillus* and *Streptococcus* species in raw HW can be eliminated after processing with extrusion.

The group consuming reference diet with casein showed significantly higher weight gain (86.5 ± 7.21g) as compared to cooked, autoclaved and extruded HW meals. The birds consuming extruded HWM showed less weight gain (72.22 ± 6.21 g) as compared to those of cooked and autoclaved. The value of PER in extruded group was significantly less, possibly because of high temperature and pressure experienced HW during extrusion. Batterham *et al.* (1986) reported in their study that an increase in processing temperature from 125°C to 150°C can cause reduction in lysine availability from 86 to 35%, which is a very essential amino acid for broiler growth. The results were supported by the findings of Barbour *et al.* (1995) who found reduction in feed intake and PER when diets containing 48% SBM were decreased from 20 to 16%. The PER results from all processing techniques along with NPU data supported an over all conclusion that processing HW with cooking and autoclaving was quite comparable in terms of NPU but significant difference was due to harsh temperature and pressure. It is notable that autoclaved proved more beneficial in terms of PER. Hackler *et al.* (1984) showed that irrespective of the species, other factors like protein source, interaction of sources and level of protein may be the reason for the difference in PER. But overall values of PER and NPU reveal that processing of HWM can generate nutrient rich, palatable ingredients that are comparable to the traditional ingredients for better broiler performance. PER showed good results in terms of protein quality on all test diets. Casein produced better protein efficiency in chicks. Michele *et al.* (1997) used one source of protein *i.e.,* spent hen meal for determination of PER. They arranged spent hen meal by three different processing techniques that is why there was disparity in PER of meal.

In conclusion it can safely be said that HWM can be used after different treatments without much losing their protein quality.

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